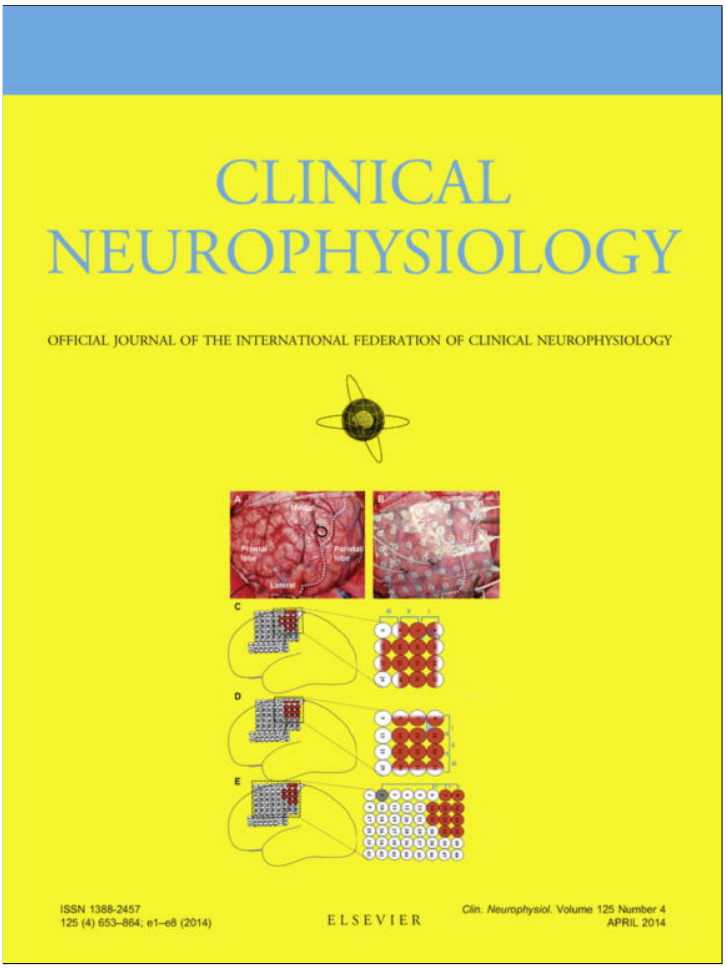

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A study of adaptation mechanisms based on ABR recorded at high stimulation rate

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HIGHLIGHTS

- The fast and slow adaptation mechanisms are studied for the first time in humans through the separated responses methodology.
- Both fast and slow mechanisms of adaptation are present in all subjects, which is consistent with previous animal studies based on spike rate.
- The morphology of the ABR is not only influenced by the stimulation rate, but also by the distribution of the jitter, and by the sequencing of stimuli.

ABSTRACT

Objective: This paper analyzes the fast and slow mechanisms of adaptation through a study of latencies and amplitudes on ABR recorded at high stimulation rates using the randomized stimulation and averaging (RSA) technique.
Methods: The RSA technique allows a separate processing of auditory responses, and is used, in this study, to categorize responses according to the interstimulus interval (ISI) of their preceding stimulus. The fast and slow mechanisms of adaptation are analyzed by the separated responses methodology, whose underlying principles and mathematical basis are described in detail.
Results: The morphology of the ABR is influenced by both fast and slow mechanisms of adaptation. These results are consistent with previous animal studies based on spike rate.
Conclusions: Both fast and slow mechanisms of adaptation are present in all subjects. In addition, the distribution of the jitter and the sequencing of the stimuli may be critical parameters when obtaining reliable ABRs.
Significance: The separated responses methodology enables for the first time the analysis of the fast and slow mechanisms of adaptation in ABR obtained at stimulation rates greater than 100 Hz. The non-invasive nature of this methodology is appropriate for its use in humans.

1. Introduction

Adaptation of the auditory system is a decrease in response when a maintained stimulus or successive click stimuli are presented (Thornton and Coleman, 1975; Gillespie and Muller, 2009). Modeling of adaptation has unleashed controversy since Sorensen (1959) postulated that the decrease in the response could be associated with either a decrease in the number of active nerve fibers, or a decrease of their spike rate. Later, other authors suggested that the mechanisms of adaptation not only comprise the synapses of hair cells, but also the axonal transmission characteristics of the neurons that compose the auditory nerve (e.g., Chimento and Schreiner, 1991; Woo et al., 2009). Most of the authors agree on the combination of various types of mechanisms involved in the adaptation process whose effects are manifested in

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different time scales: fast adaptation occurs during the first few milliseconds following stimulus onset, whilst slow adaptation is about ten-fold slower (around 40–100 ms) (e.g., Eggermont, 1985; Yates et al., 1985; LeMasurier and Gillespie, 2005; Zhang et al., 2007). Slower effects of adaptation (up to several seconds after the beginning of stimulation) have also been analyzed by other authors (e.g., Javel, 1996). Although the relationship between these types of adaptation is still unclear, recent studies provide different models for fast and slow adaptation. Both physiological models conclude that adaptation reduces the opening of transduction channels in cochlear hair cells, limiting the flow of K^+ and Ca^{2+} ions into the hair cell and therefore, reducing the probability of action potential generation (LeMasurier and Gillespie, 2005; Stauffer et al., 2005; Gillespie and Muller, 2009). A better understanding of the properties of adaptation in the auditory nerve may be useful for several clinical applications such as detecting certain peripheral lesions (e.g., acoustic neuroma) at an early stage or modeling the mechanotransduction process (conversion of a mechanical stimulus into an electrical response) (e.g., Don et al., 1977; Stockard et al., 1977; Yagi and Kaga, 1979).

The most relevant methods proposed to examine the effects of adaptation are based on spike rate of the auditory nerve, on otoacoustic emissions (OAE), and on auditory brainstem response (ABR). The spike rate of hair cells can be measured in animals by microelectrodes inserted into the nerve fibers and in cochlear implant patients. Many studies have characterized adaptation as a decrease in spike rate when continuous stimulation is presented. These studies report different types of adaptation according to their temporal effect following stimulus onset: rapid adaptation (few milliseconds), short-term adaptation (tens of milliseconds), long-term adaptation (seconds), and very-long-term adaptation (minutes) (Westerman and Smith, 1984; Eggermont, 1985; Yates et al., 1985; Chimento and Schreiner, 1991; Javel, 1996). The recovery time from adaptation in auditory nerve fibers has also been defined in terms of spike rate (Young and Sachs, 1973; Yates et al., 1985).

The non-invasive nature of the OAE and ABR methods makes them appropriate to study adaptation in humans. On one hand, the effects of adaptation in evoked otoacoustic emissions are manifested as a decrease in the amplitude of the response (e.g., Picton et al., 1993; Thornton, 1993; Lina-Granade and Collet, 1995; Hine et al., 2001). On the other hand, amplitudes of ABR waves decrease and latencies increase as a consequence of adaptation, especially in more central response components (e.g., Thornton and Coleman, 1975; Yagi and Kaga, 1979; Lasky, 1984; Jiang et al., 2009; Valderrama et al., 2012a).

Conventional ABR recording technique consists of averaging several auditory responses whose corresponding stimuli are presented periodically. Many studies have used the conventional recording technique to analyze the effects of adaptation in ABR (e.g., Thornton and Coleman, 1975; Yagi and Kaga, 1979; Lasky, 1997, 1984; Polyakov and Pratt, 2003; Jiang et al., 2009). Some of these studies presented trains of clicks and recorded the transition from unadapted ABR to adapted ABR. The conventional technique has the limitation that the inter-stimulus interval (ISI) must be greater than the averaging window in order to avoid the contamination of the recording by the adjacent response (e.g., Kjaer, 1980). Thus, the conventional technique cannot be used to record ABR at rates higher than 100 Hz, considering a standard averaging window of 10 ms. However, the use of higher stimulation rates allows a more detailed study of adaptation since its effects increase with stimulus duration, stimulus level, and stimulation rate (e.g., Killian et al., 1994; Burkard et al., 1996a,b; Haenggeli et al., 1998; Clay and Brown, 2007; Zhang et al., 2007).

It is not mathematically possible to recover the overlapped ABR signal when the stimulation sequence is periodic (conventional stimulation) (e.g., Jewett et al., 2004). On this framework, different techniques have emerged to overcome the limitation imposed by the conventional technique. These techniques are able to obtain the superposed ABR signal using jittered stimuli (the *jitter* of a stimulation sequence measures the grade of dispersion of the ISI in contrast to a periodical presentation of stimuli where ISI would be constant). The most relevant techniques developed to record ABR at stimulation rates higher than 100 Hz are maximum length sequences (MLS) (Eysholdt and Schreiner, 1982), continuous loop averaged deconvolution (CLAD) (Delgado and Ozdamar, 2004; Ozdamar and Bohorquez, 2006), quasi-periodic sequence deconvolution (QSD) (Jewett et al., 2004), and randomized stimulation and averaging (RSA) (Valderrama et al., 2012a). The MLS technique has been widely used to explore the effects of adaptation in ABR recorded at high stimulation rates (e.g., Thornton and Slaven, 1993; Burkard et al., 1996a,b; Leung et al., 1998; Lavoie et al., 2008). The MLS, CLAD, and QSD techniques obtain the auditory response by averaging a number of blocks of responses corresponding to a predefined stimulation sequence, and then, deconvolving the response from the stimulation sequence by different procedures. The influence of the distribution of the jitter on the morphology of the auditory responses has not already been analyzed because the techniques based in deconvolution assume the premise that each click evokes the same response. The ABR recorded with RSA is obtained directly by averaging the responses after applying a digital blanking process which is useful for minimizing the effect of stimulation artifact. In comparison to CLAD, and QSD, the RSA technique allows a precise control of the jitter of the stimulation sequence, and a separate processing of auditory responses.

This article presents a study of the fast and slow adaptation mechanisms based on ABR obtained with the RSA technique. Portions of this research were presented at the Adult Hearing Screening Congress, Cernobbio (Lake Como), Italy, June 7–9, 2012 (Valderrama et al., 2012b). The present study compares the amplitudes and latencies of waves III and V of the ABR obtained in different recording conditions. The stimulation sequences considered in this study are: (a) stimulation sequences with jitter distributions of long ISIs, (b) of short ISIs, and (c) of both long and short ISIs randomly distributed. The auditory responses corresponding to the long-and-short ISIs stimulation sequence were categorized according to the ISI of their preceding stimulus (long or short), and two ABR signals were obtained using these categories. If the morphology of the ABR-L and ABR-S signals (i.e. average of responses who's preceding ISIs were long and short, respectively) were similar, that would suggest that the adaptation responds to slow mechanisms since the morphology of the ABR depends in a great extent on the stimulation rate of several preceding stimuli. On the other hand, if the morphology of ABR-L and ABR-S were similar to their corresponding ABR signals recorded with long and short ISI stimulation sequences, that would mean that the adaptation responds to fast mechanisms because the morphology of the response is strongly influenced by the ISI of the preceding stimulus. The results of this experiment show that most of the subjects analyzed in the study give results that lie in between both described situations, which suggests that both fast and slow mechanisms are involved in the adaptation process. The relevance of these findings is discussed in this article.

2. Methods

2.1. Subjects

Eighteen subjects with no self-reported history of auditory dysfunction (normally hearing subjects), 4 females and 14 males, aged from 25 to 62 years (with a mean age of 34 years) participated in

this study. These subjects were chosen randomly from different social sectors from the University of Granada (e.g., students, professors, etc.). All subjects were volunteers and were informed about the experimental protocol and possible side effects of the test. A consent form was signed by the participants before the beginning of the recording session, which was carried out at the University of Granada (Granada, Spain) accordingly to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. This recording procedure was approved by the Human Research Ethics Committee of the University of Granada and by the Clinical Research Ethics Committee of the San Cecilio University Hospital.

2.2. EEG recording

The subjects were stimulated by clicks at an intensity of 70 dB above normal hearing level threshold (dBnHL). Monophasic clicks of 0.1 ms in condensation polarity were chosen as stimuli to evoke a synchronous firing of a large number of neurons, in particular those in the 1000–4000 Hz region (Hall, 2007; Thornton, 2007). The recording sessions were held in a room prepared to attenuate acoustical and electromagnetic interference. The subjects were seated in a comfortable position during the recording session in order to minimize the electromyogenic noise. Standard circumaural headphones (Pro-550, Ultrasone, Wielenbach, Germany) were used to present the stimuli to the subjects. The auditory evoked responses were recorded by three Ag/AgCl surface electrodes placed on the skin at the high forehead (active), ipsilateral mastoid (reference), and low forehead (ground). Interelectrode impedances were below 10 kΩ in all recordings. The signal recorded by the electrodes was amplified and band-pass filtered (100–3500 Hz). The band limits of the filters were chosen to maximize the detectability of all waves (Thornton, 2007). The synchronization of the biological signal with the stimuli was achieved through a synchronous recording of the EEG and the stimulation signal by a two-channel analogue-to-digital converter. Signals were sampled at 25 kHz and stored using 16 bits/sample. Data processing was carried out by algorithms implemented in MATLAB (The Mathworks, Inc., Natick, MA). The recorded EEG was digitally filtered using a sixth order bandpass Butterworth filter (150–3000 Hz). A full description of the ABR recording system can be found in Valderrama et al. (2011).

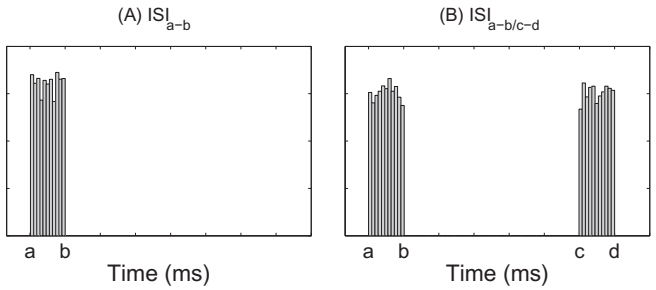

Fig. 1. Distribution of the jitter for the two types of stimulation sequences used in this study. (A) Histogram of the interstimulus interval (ISI) for an ISI_{a-b} stimulation sequence: the ISI varies uniformly random within the interval [a,b] ms. (B) Histogram of the ISI for an $ISI_{a-b/c-d}$ stimulation sequence: the ISI varies uniformly random within the intervals [a,b] and [c,d] ms.

2.3. ABR obtained with RSA

The recording of ABR at high stimulation rates using the RSA technique is appropriate to analyze the effects of adaptation. The ABR signal is obtained in RSA by averaging auditory responses corresponding to stimuli whose ISI varies randomly according to a

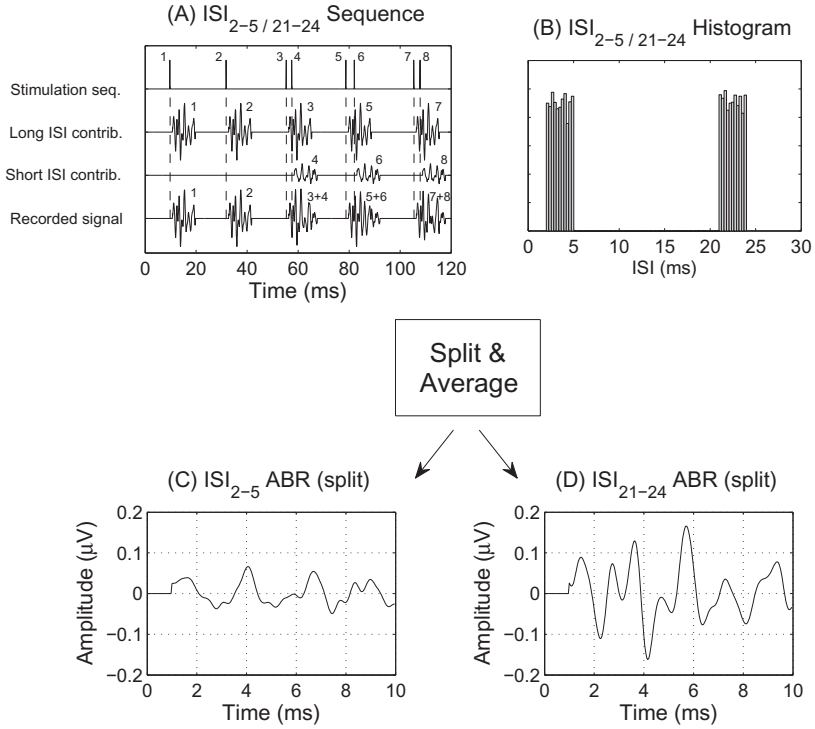

Fig. 2. Scheme of the process of separated responses. (A) Frame of an $ISI_{2-5/21-24}$ stimulation sequence. The auditory response contribution without noise from each stimulus is categorized according to their preceding ISI. The stimuli and their associated auditory responses are numerated. The "Long ISI contrib." and "Short ISI contrib." signals shows the auditory responses corresponding to the stimuli whose preceding ISI belong to the intervals [21,24] and [2,5] ms, respectively. The "Recorded signal" shows the sum of both long and short ISI ABR contributions. (B) Histogram of the interstimulus interval for an $ISI_{2-5/21-24}$ stimulation sequence. (C) ABR obtained with the auditory responses whose preceding ISI belong to the interval [2,5] ms. (D) ABR obtained with the auditory responses whose preceding ISI belong to the interval [21,24] ms.

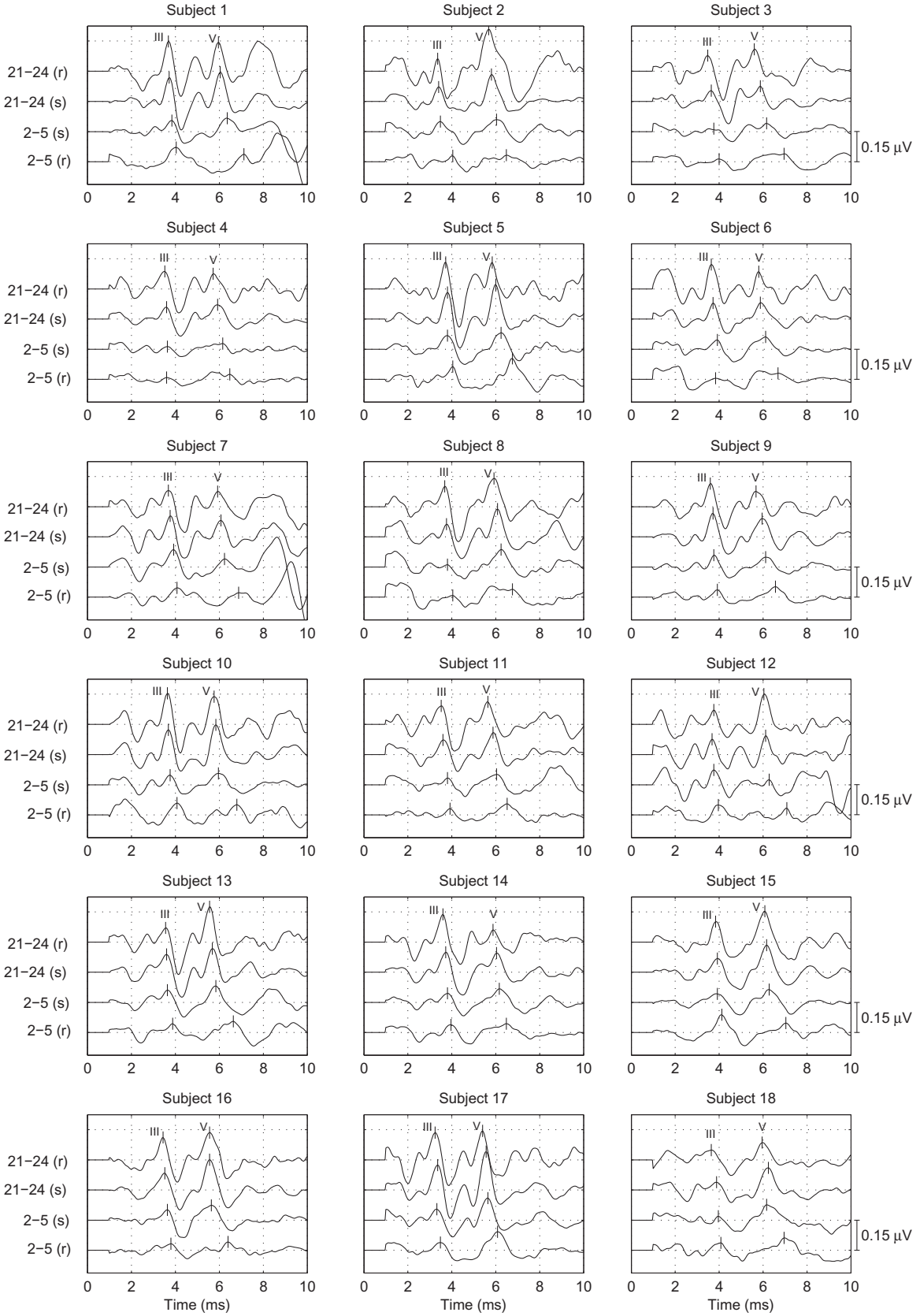

Fig. 3. ABR signals from 18 subjects obtained in the following recording conditions. The '21–24 (r)' and '2–5 (r)' signals represent the recorded ABRs obtained using the randomized stimulation and averaging (RSA) technique with the stimulation sequences ISI_{21-24} and ISI_{2-5}, respectively. The '21–24 (s)' and '2–5 (s)' represent the separated ABRs obtained with the separated responses methodology on the stimulation sequence $ISI_{2-5/21-24}$. Waves III and V are identified in all recordings.

Table 1
Interval and mean (standard deviation in parentheses) of the latencies (L) and amplitudes (A) of the waves III and V from the ABR signals presented in the Fig. 3. Latencies and amplitudes are measured in milliseconds and microvolts, respectively. This table shows that the averaged amplitudes and latencies of the separated '21–24 (s)' and '2–5 (s)' ABR signals are in between their corresponding ABR recorded signal and their opposite separated ABR signal.

	21–24 (r)		21–24 (s)		2–5 (s)		2–5 (r)	
	Interval	Mean (S.D.)	Interval	Mean (S.D.)	Interval	Mean (S.D.)	Interval	Mean (S.D.)
L_{III} (ms)	[3.24 3.86]	3.60 (0.15)	[3.36 3.94]	3.68 (0.15)	[3.32 3.96]	3.77 (0.17)	[3.48 4.10]	3.93 (0.16)
L_V (ms)	[5.40 6.08]	5.79 (0.19)	[5.56 6.20]	5.93 (0.19)	[5.64 6.36]	6.11 (0.21)	[6.12 7.12]	6.72 (0.28)
A_{III} (μV)	[0.13 0.32]	0.23 (0.05)	[0.10 0.28]	0.18 (0.05)	[0.05 0.15]	0.11 (0.03)	[0.04 0.15]	0.08 (0.03)
A_V (μV)	[0.12 0.36]	0.19 (0.06)	[0.12 0.26]	0.17 (0.04)	[0.05 0.16]	0.10 (0.03)	[0.05 0.17]	0.08 (0.03)

Table 2
Interval, mean (standard deviation in parentheses) and *p*-value of the differences of latencies (L) and ratio of amplitudes (A) between pairs of ABR from each subject obtained in different conditions in a group of 18 subjects. This analysis suggests that all ABRs compared in this study are statistically different (p-value < 0.05) in terms of amplitudes and latencies, in exception for A_V in '21–24 (s)/21–24 (r)'.

	21–24 (s) – 21–24(r)			2–5 (s) – 21–24(s)			2–5 (r) – 2–5 (s)		
	Interval	Mean (S.D.)	p-value	Interval	Mean (S.D.)	p-value	Interval	Mean (S.D.)	p-value
L_{III} (a) – L_{III} (b) (ms)	[−0.08 0.20]	0.08 (0.06)	4×10^{-5}	[−0.04 0.34]	0.09 (0.09)	8×10^{-4}	[−0.02 0.56]	0.17 (0.13)	5×10^{-5}
L_V (a) – L_V (b) (ms)	[−0.02 0.30]	0.15 (0.08)	7×10^{-7}	[0.00 0.30]	0.18 (0.08)	2×10^{-8}	[0.30 0.84]	0.61 (0.18)	9×10^{-11}
	21–24 (s)/21–24(r)			2–5 (s)/21–24(s)			2–5 (r)/2–5 (s)		
A_{III} (a)/A_{III} (b)	[0.60 1.07]	0.82 (0.14)	7×10^{-4}	[0.36 0.93]	0.60 (0.17)	2×10^{-4}	[0.45 1.50]	0.82 (0.29)	0.020
A_V (a)/A_V (b)	[0.50 1.33]	0.95 (0.23)	0.407	[0.35 0.86]	0.60 (0.13)	$2 \cdot 10^{-4}$	[0.54 1.21]	0.81 (0.20)	0.004

predefined probability distribution. The RSA technique includes a digital blanking process and non-uniform averaging which considers as null values those samples contaminated by the stimulation artifact (0.2 ms before and 0.8 ms after each stimulus). These null values are not considered in the averaging process. A basic artifact rejection technique was used to improve the quality of the recordings: auditory responses whose amplitude exceeded the range ±10 μV were not considered in the averaging process. The RSA technique is described in detail in Valderrama et al. (2012a).

The RSA technique allows a precise control of the jitter in the process of stimulation sequences generation. The stimulation sequences used in this study present two types of jitter distributions. The distribution of the jitter for each type of stimulation sequence is presented in Fig. 1. This study involves ISI_{a-b} stimulation sequences, whose ISI varies randomly with an uniform distribution within an interval 'a' to 'b' ([a,b]) ms (Fig. 1A); and $ISI_{a-b/c-d}$ stimulation sequences, whose ISI varies with a uniform random distribution between the intervals 'a' to 'b' ([a,b]) and 'c' to 'd' ([c,d]) ms (Fig. 1B).

2.4. Separated responses

The separated responses methodology is based in a separate processing of auditory responses, which can be performed using the RSA technique. Fig. 2 outlines the process of separating the responses. Fig. 2A shows a frame from an $ISI_{2-5/21-24}$ stimulation sequence and their associated auditory responses without noise. The ISI of this stimulation sequence varies with a uniform random distribution between the intervals [2,5] and [21,24] ms, as shown by its histogram in Fig. 2B. The auditory responses can be categorized according to their preceding ISI. The auditory responses whose preceding ISI belong to the interval [21,24] ms (associated to stimuli 1, 2, 3, 5, 7) are shown as "long ISI contribution", and those whose preceding ISI belong to the interval [2,5] ms (associated to stimuli 4, 6, and 8) are shown as "short ISI contribution". The "recorded signal" in Fig. 2A shows the sum of both long and short ISI contributions. Fig. 2C and D show the ABR obtained using the RSA technique with the auditory responses that belong to each interval.

2.5. Description of the experiments

The following EEGs were recorded from each subject: 5000 auditory responses corresponding to an ISI_{21-24} stimulation sequence, 10,000 auditory responses corresponding to an $ISI_{2-5/21-24}$ stimulation sequence, and 20,000 auditory responses corresponding to an ISI_{2-5} stimulation sequence. The auditory responses were recorded, stored and processed offline. The number of recorded responses increases at higher stimulation rates because the quality of the ABR degrades as stimulation rate increases as a consequence of adaptation (e.g., Don et al., 1977; Valderrama et al., 2012a), and therefore, more auditory responses are needed in order to obtain ABR signals of similar quality. From the EEG corresponding to an $ISI_{2-5/21-24}$ stimulation sequence, two ABR signals were obtained after the separated responses procedure described in Section 2.4. Thus, these two separated ABR signals were obtained with approximately 5000 auditory responses. The amplitudes and latencies of the waves III and V were measured as a difference in milliseconds between the top of the peaks and the stimulus onset for latencies, and the amplitudes as the difference in microvolts between the top of the peak and the following trough (Thornton, 2007; Hall, 2007).

The mean and standard deviation of the amplitudes and latencies were calculated among the 18 subjects. The separated ABR responses and the recorded ABR responses were compared in terms of latencies by a matched paired *t*-test and in terms of amplitudes by a matched paired Wilcoxon signed rank test. Two hypotheses are considered in this study: (1) the recorded ISI_{21-24} ABR is similar to the separated ISI_{21-24} ABR and the recorded ISI_{2-5} ABR is similar to the separated ISI_{2-5} ABR (the two separated ABRs are different); and (2) both separated ISI_{21-24} and ISI_{2-5} ABRs are similar. On one hand, hypothesis 1 would indicate that the auditory system adapts according to fast mechanisms since the morphology of the separated ABR would be very much influenced by the ISI of the preceding stimulus. On the other hand, hypothesis 2 would suggest that adaptation is a slow process which is mostly influenced by the stimulation rate of several preceding stimuli (the influence of the preceding stimulus is not determinative).

This paper also includes a study that analyzes the effect of the slow mechanisms of adaptation on the morphology of the

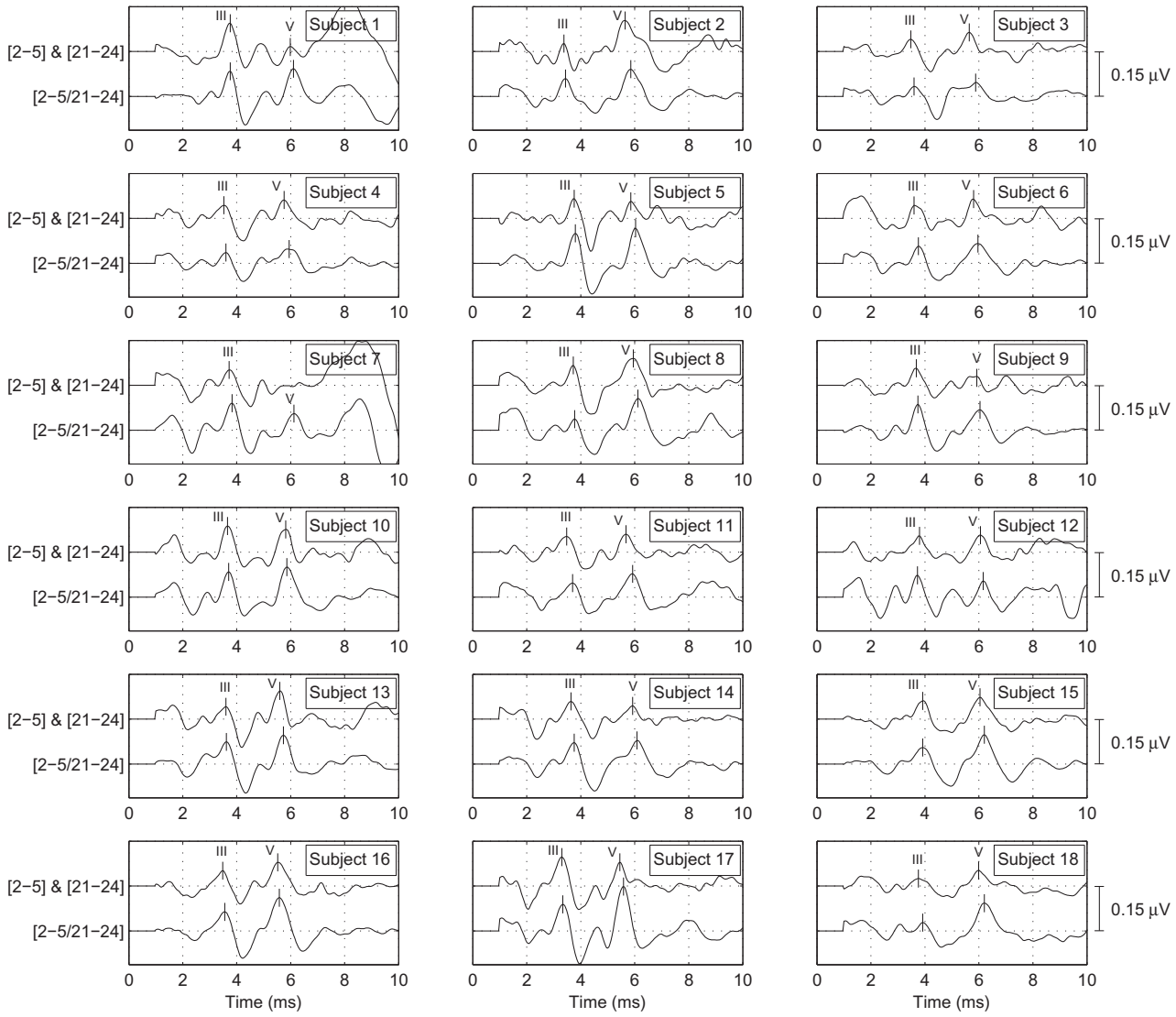

Fig. 4. ABR signals from 18 subjects obtained using 10,000 auditory responses from stimulation signals of equal distributions of the jitter but different order of presentation of stimuli: whilst the ISI in the [2–5/21–24] stimulation sequence vary uniformly random between the ranges [2,5] and [21,24] ms all along the stimulation sequence, the ISI in the [2–5] and [21–24] stimulation sequence vary uniformly random between the range [21–24] ms during the first 5000 stimuli, and between the range [2,5] ms during the last 5000 stimuli. Waves III and V are labeled in the figure.

ABR. ABRs from 18 subjects obtained with 10,000 stimuli from an $ISI_{2-5/21-24}$ stimulation signal (ABR [2–5/21–24]) were compared to ABRs obtained by averaging 5000 auditory responses from an ISI_{21-24} stimulation sequence and 5000 auditory responses from an ISI_{2-5} stimulation sequence (ABR [2–5]&[21–24]). These two ABRs are obtained with stimulation sequences of the same distribution of the jitter, but a different sequencing of stimuli. On the ABR [2–5/21–24], the ISI of the stimuli varies uniformly random between the ranges [2,5] and [21,24] ms all along the stimulation sequence; whilst on the ABR [2–5]&[21–24], the ISI varies uniformly random between [21,24] ms during the first 5000 stimuli and between [2,5] ms during the last 5000 stimuli. Considering that the fast mechanisms of adaptation are manifested within the few milliseconds following stimulus onset, these two ABR signals are influenced in the same manner by the fast mechanisms of adaptation since both ABRs involve 5000 auditory responses whose preceding ISI belong to the interval [2,5] ms and 5000 responses whose preceding ISI belong to the interval [21,24] ms. The two ABR signals of this experiment will be different according to the effects of the slow mechanisms of adaptation. The slow mechanisms of adaptation are manifested, on one hand, during 10,000 responses at an averaged ISI of 13 ms on the ABR [2–5/21–24]; and on the other hand, at an averaged ISI of 22.5 ms during the first 5000 responses and at an averaged ISI of 3.5 ms during the last 5000 responses on the ABR [2–5]&[21–24]. A statistical difference among the two ABR signals obtained with this experimental protocol could be used to detect the influence of the slow mechanisms of adaptation on the ABR.

3. Results

Fig. 3 shows ABR signals obtained from the group of 18 subjects in the previously described recording conditions. The recorded ABR signals corresponding to the ISI_{21-24} and ISI_{2-5} stimulation sequences are represented by '21–24 (r)' and '2–5 (r)', respectively; and the separated ABR signals are represented by '21–24 (s)' and '2–5 (s)'. The waves III and V are labeled in the figure and were identified in all subjects. Despite the differences in the morphology among ABR from different subjects, this figure shows that most of

the subjects present a similar pattern, which is analyzed in Tables 1 and 2.

Table 1 shows the mean and standard deviation of the latencies and amplitudes of waves III and V in a group of 18 subjects. The amplitudes and latencies measured on the recordings '21–24 (r)' and '2–5 (r)' are consistent with previous literature (e.g., Yagi and Kaga, 1979; Lasky, 1984; Lina-Granade et al., 1993; Leung et al., 1998; Jiang et al., 2009; Valderrama et al., 2012a,b). This table indicates that both amplitudes and latencies are influenced by the stimulation rate: amplitudes decrease and latencies increase as stimulation rate increases as a consequence of adaptation. The effects of adaptation in latencies are more remarkable in wave V than in wave III, since the stimulation rate influences in a greater extent those components generated in a more central site (e.g., Pratt and Sohmer, 1976; Yagi and Kaga, 1979; Jiang et al., 2009; Valderrama et al., 2012a). This table shows that, on average, the recorded '21–24 (r)' ABR signals present greater amplitudes and lower latencies than the '2–5 (r)' signals in both waves; that the separated '21–24 (s)' ABR signal presents amplitudes and latencies in between the '2–5 (s)' and the '21–24 (r)' ABR signals; and that the '2–5 (s)' ABR signal presents amplitudes and latencies in between the '21–24 (s)' and the '2–5 (r)' ABR signals.

Table 2 compares the amplitudes and latencies of waves III and V from pairs of ABRs from each subject and analyzes whether or not their differences are statistically significant. The latencies and amplitudes are analyzed in this table in terms of differences and ratio, respectively. Table 2 shows the mean and standard deviation of the differences of latencies and ratio of amplitudes between the following pairs of ABRs: '21–24 (r)' vs '21–24 (s)', '21–24 (s)' vs '2–5 (s)', and '2–5(r)' vs '2–5 (s)'. The p-value shown in the table indicates the probability of obtaining those results by chance, considering as reference differences of latencies equal to zero and ratio of amplitudes equal to one. The large standard deviation of these parameters points out a large variability among subjects. This table also shows that there are statistically significant differences (p-value < 0.05) between (a) both separated '21–24 (s)' and '2–5 (s)' ABR signals in terms of amplitudes and latencies, and (b) between each separated ABR signals and its corresponding recorded ABR signals. The morphology of the recordings '21–24 (s)' and '21–24 (r)' may be assumed to be different despite their ratio of A_V does not show statistically significant differences (p-value > 0.05), since the rest of parameters (A_{III}, L_{III}, and L_V) are statistically different.

Fig. 4 shows ABR signals from 18 subjects corresponding to a stimulation sequence in which the ISI varies uniformly random between the ranges [2,5] and [21,24] ms (shown as '2–5/21–24' in the figure); and corresponding to a stimulation sequence in which the ISI vary between the interval [21,24] ms during the first 5000 stimuli, and between the interval [2,5] ms during the last 5000 stimuli (shown as '[2–5]&[21–24]' in the figure). The mean and standard deviation of the amplitudes and latencies of the waves III and V in these recordings are shown in Table 3. Waves III and V could be identified in all signals, in exception for the wave V in subject 7 in the [2–5]&[21–24] ABR signal. The two ABR recordings from each subject were compared with a matched paired t-test for differences of latencies and with a matched Wilcoxon signed rank test for ratio of amplitudes. The analysis for waves III and V were made with 18 and 17 subjects, respectively. The results of this study are presented in Table 4. This table shows that there are statistically significant differences between the '[2–5/21–24]' and the '[2–5]&[21–24]' ABR signals, which confirms the influence of the slow mechanisms of adaptation on the morphology of the auditory response.

Table 3
Interval and mean (standard deviation in parentheses) of the latencies (L) and amplitudes (A) of waves III and V from the ABR signals presented in Fig. 4.

	[2–5/21–24]		[2–5] & [21–24]	
	Interval	Mean (S.D.)	Interval	Mean (S.D.)
L_{III} (ms)	[3.36 3.92]	3.70 (0.15)	[3.28 3.94]	3.63 (0.17)
L_V (ms)	[5.58 6.20]	5.96 (0.19)	[5.44 6.04]	5.80 (0.18)
A_{III} (μV)	[0.08 0.20]	0.14 (0.04)	[0.06 0.18]	0.12 (0.03)
A_V (μV)	[0.06 0.21]	0.13 (0.04)	[0.04 0.17]	0.09 (0.03)

Table 4
Interval, mean (standard deviation in parentheses) and p-value of the differences of latencies (L) and ratio of amplitudes (A) between pairs of ABR from each subject obtained in different recording conditions. This table remarks that there are statistically significant differences between the [2–5/21–24] and the [2–5]&[21–24] ABR signals (p-value < 0.05).

	[2–5/21–24] – [2–5]&[21–24]		
	Interval	Mean (S.D.)	p-value
L_{III} (a) – L_{III} (b) (ms)	[−0.08 0.24]	0.07 (0.07)	$6{\cdot}10^{-4}$
L_V (a) – L_V (b) (ms)	[0.04 0.26]	0.16 (0.05)	$9{\cdot}10^{-10}$
	[2–5/21–24]/[2–5]&[21–24]		
		Mean (S.D.)	p-value
A_{III} (a)/A_{III} (b)	[0.75 2.00]	1.24 (0.29)	0.003
A_V (a)/A_V (b)	[0.60 3.00]	1.57 (0.69)	0.003

4. Discussion

This article presents a study of the fast and slow mechanisms of adaptation based on ABR signals obtained at high stimulation rates using the RSA technique. The recorded '21–24 (r)' and '2–5 (r)' ABR signals were obtained using directly the RSA technique with auditory responses whose ISI varied randomly within the range [21,24] and [2,5] ms, respectively. The separated '21–24 (s)' and '2–5 (s)' ABR signals were obtained using the separated responses methodology with the EEG corresponding to the $ISI_{2-5/21-24}$ stimulation sequence, which allows the retrieval of auditory responses whose preceding ISI belong to the interval [21,24] ms or to the interval [2,5] ms. The comparison of ABR signals was carried out by an analysis of the differences in latencies and ratio of amplitudes. If the separated ABR signals were similar to their corresponding recorded ABR signals (both separated ABRs were different), the fast mechanisms of adaptation would prevail over the slow mechanisms since the morphology of the response would be influenced in a greater extent by the ISI of the preceding stimulus. On the other hand, if the separated ABR signals were different to their corresponding recorded ABR signals and both separated ABRs were similar, the slow mechanisms of adaptation would have prevailed over the fast mechanisms because the morphology of the response would be mainly determined by the averaged stimulation rate of several preceding stimuli (but not by the ISI of the preceding stimulus).

The results of this study indicate that most of the subjects present a situation in between both hypotheses, which suggests that both fast and slow mechanisms of adaptation influence the morphology of the auditory response. There exists a great variability among subjects (Fig. 3). For instance, the separated ABR signals in subjects 10 and 17 present high differences in amplitudes but small differences in latencies; subjects 1 and 5 present high differences in both amplitudes and latencies; and subjects like 15 and 16 show small differences in amplitudes but high differences in latencies. On average, the latencies and amplitudes of the main waves in the '21–24 (s)' and '2–5 (s)' ABR signals are, respectively, in between the '2–5 (s)' and the '21–24 (r)' ABR signals on one hand, and between the '21–24 (s)' and the '2–5 (r)' on the other hand (see Table 1). The results presented in Table 2 show that the two separated ABR signals are statistically different, and that there

are statistically significant differences between the separated ABR responses and their corresponding recorded ABR responses (see Table 2). These findings indicate that the morphology of the separated ABR is influenced both by the ISI of the preceding stimulus and by the averaged stimulation rate of several preceding stimuli, which suggests that both fast and slow mechanisms are involved in the adaptation process.

This paper also includes an experimental protocol to detect the influence of the slow mechanisms of adaptation on the morphology of ABR. The results presented in Fig. 4 and Tables 3 and 4 show statistically significant differences between ABRs obtained with long and short ISI clicks randomly presented all along the stimulation sequence (averaged ISI of 13 ms) and ABRs obtained with long ISI clicks in the beginning (averaged ISI of 22.5 ms) and short ISI clicks in the end (averaged ISI of 3.5 ms). These results confirm the existence of slow mechanisms of adaptation in ABR. In addition, these results indicate that the morphology of the ABR is not only influenced by the average stimulation rate, but also by the distribution of the jitter and the sequencing of the stimuli.

The results presented in this paper are consistent with previous studies, in which the fast and slow mechanisms of adaptation are characterized in animals in terms of spike rate (e.g., Westerman and Smith, 1984; Eggermont, 1985; Yates et al., 1985; Javel, 1996). The fast mechanisms of adaptation analyzed in this study are manifested during the first few milliseconds following stimulus onset and may be related to the rapid adaptation described in Westerman and Smith (1984) and in Yates et al. (1985). Although the time constant for the slow mechanisms of adaptation is not determined in this paper, the results presented in Fig. 4 and Tables 3 and 4 indicate that the time constant for the slow mechanisms of adaptation might be greater than 20 ms, otherwise the effects of slow adaptation would not have been observed in that experiment. The slow mechanisms of adaptation observed in these experiments may be related to the short-term adaptation defined in Westerman and Smith (1984) and to the long-term adaptation described in Javel (1996), whose time constant varies from several tens of milliseconds to a few seconds.

The non-invasive nature of the process of ABR recording is appropriate to study the effects of adaptation in humans. Traditionally, the adaptation of the hearing system was analyzed by presenting to the subject trains of stimuli of a fixed ISI, and comparing the morphology of the ABRs corresponding to each position in the train (e.g., Thornton and Coleman, 1975; Lasky, 1997; Polyakov and Pratt, 2003). This methodology presents the limitation that the ISI must be greater than the averaging window. Thus, the adaptation cannot be studied using this methodology at rates greater than 100 Hz. Other techniques like MLS, CLAD, or QSD allow the recording of ABR at very high stimulation rates (Eysholdt and Schreiner, 1982; Delgado and Ozdamar, 2004; Jewett et al., 2004; Ozdamar and Bohorquez, 2006). These techniques obtain the ABR signal through jittered stimuli and different deconvolution processes, which require the processing of sets of responses, and therefore, limit the study of the fast and slow mechanisms of adaptation since they assume that each click evokes the same response. The separated responses methodology performed with RSA allows for the first time a separate processing of auditory responses at stimulation rates greater than 100 Hz, which can be used to study the fast and slow effects of adaptation. The flexible control of the distribution of the jitter, the design of the sequencing of stimuli, and a separate processing of auditory responses are advantages of the RSA methodology that may be of interest in the design of certain experiments in audiology.

Despite that both fast and slow mechanisms of adaptation studied in this article seem to be related to changes in the auditory mechanotransduction, the origin of such mechanisms may be analyzed separately. It is generally accepted a time boundary, at approximately 50 ms, to separate components affected by attention (endogenous components, latencies >50 ms) and those that are not (exogenous components, latencies <50 ms) (Eggermont, 2007). On one hand, the time constant for the fast mechanisms of adaptation described in this article is below 22.5 ms, which indicates that these effects may belong to mechanisms of neural adaptation. On the other hand, although the time constant for the slow mechanisms of adaptation is not specifically estimated in this work, it is definitely greater than 22.5 ms (otherwise, there would not be significant differences on the morphology between the '[2–5]&[21–24]' and the '[2–5/21–24]' ABR signals). Consequently, part of the slow mechanisms of adaptation shown in these experimental results could be associated to changes generated by central mechanisms associated to habituation (i.e., dependent on attention) (Thompson and Spencer, 1966; Groves and Thompson, 1970; Rankin et al., 2009; Thompson, 2009).

Whilst several previous studies have demonstrated that the morphology of the ABR depends on the averaged stimulation rate (e.g., Lasky, 1984; Burkard et al., 1996a,b; Jiang et al., 2009; Valderrama et al., 2012a), it has not been shown either theoretically or experimentally that any particular distribution of the jitter has any particular significance on the morphology of the ABR. This may be due to the assumption of time invariance of auditory responses by the techniques based in deconvolution (e.g., MLS, CLAD, QSD). The auditory system may present a time invariance behaviour when short interval distributions of the jitter are used. Nevertheless, the results presented in this paper show that the morphology of the ABR is not only influenced by the ISI of the preceding stimulus, but also by the stimulation rate of several preceding stimuli, by the distribution of the jitter and by the order of presentation of the stimuli. In other words, clicks from high-jittered stimulation sequences would evoke auditory responses of different morphology. Therefore, the techniques based in deconvolution should consider all these parameters, since they assume time invariance of the auditory responses.

5. Conclusions

The separated responses methodology using RSA allows for the first time a separate processing of auditory responses at rates higher than 100 Hz, which can be used to analyze the fast and slow mechanisms of adaptation in humans. Despite the great variability of results among the analyzed subjects, the results of this study suggest that both fast and slow mechanisms are involved in the adaptation process, which is consistent with previous studies performed in animals in which adaptation is characterized in terms of spike rate. The results of this paper also show that the morphology of the auditory responses is not only influenced by the averaged stimulation rate, but also by the distribution of the jitter and the sequencing of the stimuli.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.clinph.2013.06.190.

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